

Within- and between- subject variability in methadone pharmacokinetics and pharmacodynamics in methadone maintenance subjects

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Aims

To investigate within- and between-subject variability of the pharmacodynamics and pharmacokinetics of (R)- and (S)-methadone in methadone maintenance subjects at steady-state.

Methods

Six non-holder subjects were studied on three occasions at 7–16 day intervals; doses (20–170 mg/day) remained unchanged. Blood samples and pharmacodynamic data were collected 10–12 times over a 24-h inter-dosing interval. All pharmacodynamic data were expressed as the area under the end-point versus time curve. Using analyses of variance with mixed effects, best estimates were made of the ratio of between- to within-subject variation, with corresponding 95% confidence intervals (CI) for within-subject variation at the average value.

Results

Subjects were relatively consistent between occasions, whereas there was much greater between-subject variability ($P < 0.02$) for all measures. Estimates of the ratio of between- to within-subject variation ranged from 2.2–12.8 for pharmacodynamic measures, and 1.3–7.9 for pharmacokinetic parameters. For pain, total mood disturbance, withdrawal, pupil size and respiration rate, 95% CI for within-subject measures ranged ≤ 2 -fold, while this was greater for subjective direct opioid effects (4.2-fold). For CL/F of the active (R)-methadone, the variance ratio was 4.9 ($P < 0.0003$), with 95% CI for within-subject measures ranging ≤ 2 -fold. (S)-methadone CL/F demonstrated greater within-subject variability (3.4-fold), possibly contributing to a smaller (2.7; $P < 0.0003$) ratio of between- to within-subject variance.

Conclusions

Non-holder methadone maintenance treatment participants appear to respond consistently with respect to pharmacokinetics and pharmacodynamics over a 1–2 month period. Such knowledge may help prescribers to determine whether alternative dosing regimens or treatments might be more appropriate in this population.

Introduction

Methadone is the drug most widely used as substitution treatment for opioid dependence. Although it is universally administered as a racemate, the (R)-enantiomer is the pharmacologically active moiety [1] and is more effective than the racemate in suppressing withdrawal [2, 3]. Its high oral bioavailability [4] and long terminal half-life (average 48 h or greater [5, 6]) form the basis for the once-daily oral regimens that prevail in almost all methadone maintenance treatment (MMT) programmes worldwide. The main objectives of successful substitution treatment are rehabilitation and socialization of the dependent individual, decrease in needle-associated diseases and mortality, decrease or elimination of illicit drug use and decrease in crime cost by the user and to the community [7]. Randomized controlled trials of MMT programmes in different countries have shown them to largely fulfil these aims [8–11]. However, to achieve these outcomes successfully, MMT programmes need to be able to retain patients in treatment, often indefinitely.

Even in programmes with liberal dosing policies, some patients experience withdrawal symptoms and decreased methadone efficacy for part of the dosing interval. We have shown that 34% of a nonselected sample of patients regularly experienced breakthrough withdrawal during the once daily interdosing interval. These individuals were designated as nonholders, whereas the remainder were designated as holders [12]. The nonholders also reported significantly fewer subjective (pleasurable) opioid effects than holders.

Detailed pharmacodynamic/pharmacokinetic analyses of holders and nonholders over one dosing interval at steady state have shown that, in both groups, there is evidence of an inverse relationship between plasma racemic methadone concentrations and withdrawal severity [13] and total mood disturbance [14], and a direct relationship between plasma concentrations and subjective opioid effects [13]. Similar results have been reported for (R)-methadone [15, 16]. In addition, there is evidence of a very steep plasma concentration–effect relationship for withdrawal and positive opioid effects, with mean Hill slope factors of 5.4 and 5.1, respectively [13, 14]. This means that small changes in plasma methadone concentrations would translate into relatively large changes in response.

Methadone, in common with many drugs, shows considerable between-individual variability in its pharmacokinetics and pharmacodynamics. This has been reported for racemic methadone following single doses in pain patients [17] and for (R)-methadone at steady

state in MMT patients [5, 18]. This variability in pharmacokinetics has been regarded by some as limiting its more widespread use in pain treatment [19–21]. Methadone is highly bound to plasma α_1 -acid glycoprotein and its clearance to EDDP (the major quantifiable metabolite) is mediated primarily by hepatic CYP3A4 [22]. Both α_1 -acid glycoprotein and CYP3A4 are subject to wide between-individual variability, which may be influenced by genetic, environmental, or disease-related factors. Recent population pharmacokinetic analyses have examined factors that may be predictive of methadone oral clearance in maintenance patients. As a surrogate for the unbound fraction of drug, plasma α_1 -acid glycoprotein concentration has been shown to have relatively minor predictive value for the oral clearance of methadone, presumably due to between-individual variability in the expression of α_1 -acid glycoprotein variants to which methadone binds [5]. In contrast, CYP3A4 activity, as measured by midazolam oral clearance, explains 22% of the between-individual variability in the oral clearance of unbound (R)-methadone [23].

For many drugs, there is a clearer relationship between plasma concentration and effect than that for dose and effect. Plasma methadone concentration–effect relationships have been reported for postoperative analgesia [24–26], cancer pain [27], chronic noncancer pain [28–30], experimental pain [13], sedation [30], miosis [13, 31], withdrawal [13], subjective opioid effects [13] and mood disturbance [14]. Large between-subject [24–26], but lower within-subject [25, 27] variability for methadone concentrations to achieve analgesia have been reported. The magnitude of between- and within-subject variability, and how they compare, has not been studied in MMT participants for withdrawal severity, subjective opioid effects, total mood disturbance, miosis, respiratory rate and response to experimentally induced pain. Furthermore, between- and within-subject variability of methadone pharmacokinetics has received limited attention [18].

It is likely that nonholders are at greater risk than holders of seeking illicit opioids or of leaving the MMT programme altogether [32, 33]. Nonholders might be better served with alternative treatments [13], such as twice-daily methadone, 1- α -acetylmethadol (LAAM) [16], slow release oral morphine [15] or buprenorphine [34]. However, before such strategies are considered, it is important to determine if the pharmacokinetic and pharmacodynamic characteristics of an MMT participant remain relatively constant over time. For example, if withdrawal and total mood disturbance scores are high on one occasion (consistent with nonholding status),

would similar results be expected on future occasions? We postulated that, whereas there would be considerable differences between individuals, each individual would show some degree of consistency in methadone disposition and response for a range of pharmacokinetic parameters and pharmacodynamic measures. To test this hypothesis, we compared the between- and within-subject variability in a group of nonholder MMT participants.

Methods

Patients

Ethical approval was obtained from the Royal Adelaide Hospital Research Ethics Committee. Six self-reported nonholder MMT patients (one female, five male) gave written informed consent to participate. Each had been enrolled in the South Australian Public Methadone Program for at least 6 months prior to entry into the study and had no dose change greater than 15 mg during this period. Demographic details were: age: 21–46 years; weight: 45–107 kg; once daily methadone dose for each subject: subject 1: 20 mg; subject 2: 65 mg; subject 3: 140 mg; subject 4: 105 mg; subject 5: 170 mg; subject 6: 160 mg; comedications (prescribed and illicit) remained unchanged throughout the entire study period and included: benzodiazepines (four subjects); antidepressants (four subjects); cannabis (three subjects); one subject took simvastatin, quinine and naproxen; all smoked tobacco; and only one drank alcohol (less than 20 g ethanol daily). None had illicit opiates in the urine screen. Subjects were excluded from the study if they were pregnant and if they had positive HIV serology. All subjects received AUS\$250 for completing the three study occasions.

Procedures and measures

Each subject was admitted to an inpatient facility for 24 h on three separate occasions. The shortest interval between test visits was 7 days and the longest 16 days. Subjects arrived at a prearranged time before taking their usual scheduled methadone dose. A urine specimen was obtained to check for illicit drug use. An 18-gauge intravenous catheter (Insyte™, BD Medical, UT, USA) was inserted into the best available arm vein, following which the first 5 mL blood sample was collected. Several procedures were then carried out.

1 Subjective measures included the following: (i) the Methadone Symptoms Checklist (MSC), a self-reported questionnaire that includes three groups of 16 items each, indicating direct opioid effects, opioid withdrawal symptoms, and symptoms which may be

characteristic of both direct effects and withdrawal. From this, a withdrawal score, with a maximum value of 16, is obtained [12]; (ii) the Morphine Benzodrine Group (MBG) Scale of the Addiction Research Centre Inventory [35] which includes 16 items, each of which requires a yes/no response. Evidence suggests that this is a valid and reliable self-report measure of positive opioid effects; (iii) Profile of Mood States (POMS) [36] which is divided into six empirically derived subscales (anger, depression, confusion, fatigue, tension and vigour) that reflect distinct types and qualities of identifiable affective states. The Total Mood Disturbance (TMD) score (maximum 168) is derived by summing the scores from the subscales. Since the vigour subscale can have negative values, it was decided that a new Total Mood Disturbance score would be determined by subtracting the original TMD score from 168 to achieve all positive values; (iv) pain threshold was measured by an electrical stimulus applied to one ear lobe as previously described [13].

2 Objective measures included the following: (i) pupil diameter, recorded and measured with a video-taped image of the eye, under constant illumination, using standard video recording equipment [12, 13] and (ii) respiratory rate, measured by direct observation of the subject.

Subjects then ingested their usual methadone dose (racemic methadone hydrochloride oral solution 5 mg mL⁻¹; Glaxo Wellcome Australia Ltd, Boronia, Australia) under supervision. The above subjective and objective measures were repeated and 5 mL blood samples were collected at 1, 2, 3, 4, 5, 6, 7, 9, 11 and 23 h after the dose. Two additional blood samples were collected at 0.5 and 1.5 h. Subjects were permitted to smoke tobacco and consume beverages containing caffeine and were provided with all meals during each testing session.

Plasma (R)- and (S)- methadone concentration analysis

Quantification of the enantiomers of methadone in plasma was achieved using a previously validated stereoselective HPLC assay [37]. The assay was accurate and reproducible with low (LQC, 54 ng · mL⁻¹), medium (MQC, 90 ng · mL⁻¹) and high (HQC, 350 ng · mL⁻¹) quality control samples for (R)- and (S)-methadone. Inter-assay accuracy and precision (mean accuracy ± precision) were 106 ± 7% (LQC), 103 ± 4% (MQC) and 100 ± 4% (HQC) for (R)-methadone, and 103 ± 7% (LQC), 105 ± 5% (MQC) and 103 ± 6% (HQC) for (S)-methadone. Similarly, intra-assay accuracy and preci-

sion were $108 \pm 8\%$ (LQC), $106 \pm 7\%$ (MQC) and $107 \pm 4\%$ (HQC) for (R)-methadone, and $104 \pm 7\%$ (LQC), $106 \pm 6\%$ (MQC) and $112 \pm 5\%$ (HQC) for (S)-methadone. The assay was both precise and accurate at the limit of quantification ($15 \text{ ng} \cdot \text{ml}^{-1}$), with inter-assay accuracy and precision being $101 \pm 4\%$ and $104 \pm 4\%$ for (R)- and (S)-methadone, respectively. There were no interfering peaks in any of the chromatograms except in the plasma of subject 5, whose data were excluded from the pharmacokinetic analysis, as quantification of methadone was not possible.

Data analyses

The area under the plasma concentration vs. time curve (AUC) from 0 to 24 h (the interdosing interval) was calculated by the linear trapezoidal method. The apparent oral clearance (CL/F) was calculated from the expression $\text{CL}/F = \text{dose}/\text{AUC}$, where F is the oral bioavailability. To allow for comparisons across subjects, all calculations were based on (R)- or (S)-methadone concentrations normalized to 35 mg of each enantiomer (70 mg rac-methadone). Estimates of the dose-corrected maximum and minimum plasma concentrations (C_{\max} and C_{\min}) were obtained by visual inspection of the data.

For all subjects, a significant fluctuation in the pharmacodynamic measures was observed across the 24 h observation period. Thus, for each measure, the data were expressed as the area under the end-point vs. time curve (AUC) from 0 to 24 h, calculated by the trapezoidal method.

Statistical methods

An exploratory analysis was initially conducted by viewing a scatterplot of each measure vs. subject (for each variable), with an indicator for the order in which the measurements were taken. This allowed for a visual determination of whether there was an order effect on the measurements. In addition, these scatterplots allowed for a visual determination of the extent to which the variability of each variable changed proportionally with the magnitude of the values measured for each variable. If a proportional relationship was not evident, then the coefficient of variation was not a justified summary measure. As a result, a visual inspection of scatterplots led to the determination of an appropriate summary measure.

An analysis of variance (ANOVA) with mixed effects was conducted for each variable. Subject effects were considered as random as the focus of the analysis was not on the differences between individual subjects, but on the reasons for this variation. Order was included initially as a fixed effect in an ANOVA for all variables

(alongside a random subject effect). If this fixed effect was found to be significant it was retained in the model; otherwise it was removed.

To test the null hypothesis that the random effect for between-subject variation was equal to zero, we constructed an F statistic, expressed as: $F = \frac{\hat{\sigma}^2 + 3\hat{\sigma}_B^2}{\hat{\sigma}^2}$ where $\hat{\sigma}_B^2$ is the estimated variation between individuals and $\hat{\sigma}^2$ is the estimated variation within individuals (or residual variation) [38].

The probability of obtaining an F statistic like this (or more extreme) from our data, if the null hypothesis was true was then determined for each variable. F statistics were compared with an F distribution with (5,12) degrees of freedom for all ANOVAs, except that for those associated with the analysis of plasma (R)-methadone and (S)-methadone concentration-related data, where there were (4,10) degrees of freedom.

If the P -value obtained was less than 0.05, we concluded that there was statistically significant evidence against the null hypothesis. In this case we then constructed our best estimate of the ratio of variances, expressed as: $\frac{\hat{\sigma}_B^2}{\hat{\sigma}^2}$. Alternatively, if the P -value obtained

was greater than 0.05, we accepted that there was no statistically significant evidence against the null hypothesis, and the estimated ratio was not relevant.

In addition, if the null hypothesis was rejected, an approximate 95% confidence interval for measures within subjects was constructed about the grand mean using the 95% margin of error, i.e. for each variable:

$$95\% \text{ CI} \approx \text{grand mean} \pm \text{margin of error, where the margin of error} = \pm 1.96\sqrt{\hat{\sigma}^2}.$$

Exploratory statistical analysis

Visual inspection of scatterplots of measures vs. subject (for each variable) suggested that there was no order effect, with the exception of (R)-methadone AUC. In addition, with the possible exception of (R)-methadone CL/F , and (R)- and (S)-methadone C_{\min} , variability did not appear to increase proportionally with the magnitude of the measure, indicating that the ratio of variance estimates is a more appropriate summary measure for the majority of variables. However, even for these possible exceptions, it was not convincing that a coefficient of variation was entirely appropriate. As a consequence, estimating between- and within-subject variability using a coefficient of variation could afford a misleading interpretation of the comparative variability.

In an exploratory analysis, ANOVAs with mixed effects were performed for all variables including a ran-

dom effect for subject and a fixed effect for order. The results for all analyses suggested that order was statistically nonsignificant, with the exception of those for (R)-methadone C_{max} and (R)-methadone CL/F. Consequently, in the final analyses for these variables, the fixed effect of order was retained and for all others it was removed. In all cases there was evidence that the random effect for subject differences was statistically significantly different from zero (Table 1).

Results

Values for all of the variables examined, subjects were relatively consistent between occasions (Table 1), with the possible exception of (S)-methadone CL/F and direct opioid effects. In contrast, there was much greater between-subject variability, as is evident from the best variance ratio estimate (Table 1).

Figure 1 shows the relatively small within-subject and much greater between-subject variability in the CL/F for (R)-methadone for the five individuals in whom this could be measured. Between-subject variability was significantly greater (up to nearly 8-fold) than within-subject variability for all pharmacokinetic parameters (Table 1). The smallest variance ratio was obtained for C_{max} , and was similar for both (R)-methadone (1.5) and (S)-methadone (1.3). (R)-methadone displayed consistently less within-subject variability in all pharmacokinetic parameters (<2.2-fold 95% CI at the grand mean), whereas this ranged from 2 to 3.4-fold for (S)-methadone.

The withdrawal score AUC for all individuals on each occasion is shown in Figure 2. The results indicate that there is relatively small within-subject variability for all patients, except subject 4, whose AUC varied approxi-

Table 1

Results of the analysis of between-subject and within-subject variability in methadone pharmacodynamics and pharmacokinetics in MMT patients studied on three occasions

Variable	F statistic	P-value	Variance ratio*	95% margin of error for within-subject measures†	Grand mean (95% CI)‡
<i>Pharmacokinetic variables (n = 5)</i>					
(R)-MD AUC†† (ng mL ⁻¹ h)	24.64	3.7×10^{-5}	7.9	613	2877 (2264, 3490)
(R)-MD CL/F‡‡ (L h ⁻¹)	15.55	0.0003	4.9	3.5	13.4 (9.9, 16.8)
(R)-MD C_{min} §§ (ng mL ⁻¹)	13.32	0.0005	4.1	33	89 (56, 121)
(R)-MD C_{max} ¶¶ (ng mL ⁻¹)	5.50	0.0132	1.5	44	174 (130, 218)
(S)-MD AUC†† (ng mL ⁻¹ h)	21.40	6.9×10^{-5}	6.8	1014	3210 (2195, 4224)
(S)-MD CL/F‡‡ (L h ⁻¹)	9.12	0.0023	2.7	7.3	13.4 (6.1, 20.7)
(S)-MD C_{min} §§ (ng mL ⁻¹)	18.19	0.0001	5.7	43	89 (46, 131)
(S)-MD C_{max} ¶¶ (ng mL ⁻¹)	4.89	0.0191	1.3	89	209 (120, 298)
<i>Pharmacodynamic variables§ (n = 6)</i>					
Pain (score h)	39.53	4.7×10^{-7}	12.8	109	892 (783, 1001)
Total mood disturbance (score h)	24.0	7.3×10^{-6}	7.7	511	3078 (2567, 3589)
Respiration rate (breaths min ⁻¹ hr)	19.04	2.4×10^{-5}	6.0	58	324 (266, 382)
Pupil size (mm h)	13.36	0.0002	4.1	20	127 (107, 147)
Withdrawal¶¶ (score h)	8.68	0.0011	2.6	66	201 (135, 266)
Direct subjective opioid effect** (score h)	7.51	0.0021	2.2	36	59 (23, 95)

(R)-MD = (R)-methadone (S)-MD = (S)-methadone. *Best estimate of the ratio of between- to within-subject variability; †Estimated margin of error corresponding to a 95% confidence interval for within-subject measures, note that this is a constant interval and is independent of the magnitude of the measure; ‡Mean of all values in the data set with approximate 95% confidence interval for within subject measures; §Variable is reported as the area under the effect-time curve during a single interdosing interval; ¶Variable measured by the methadone symptom checklist; **Variable measured by the Morphine Benzidine Group Scale of the Addiction Research Centre Inventory; ††Area under the plasma concentration-time curve corrected to 35 mg of the respective enantiomer; ‡‡CL/F = the apparent oral clearance; §§ C_{min} = minimum steady-state plasma methadone concentration corrected to 35 mg of the respective enantiomer; ¶¶ C_{max} = maximum steady-state plasma concentration corrected to 35 mg of the respective enantiomer.

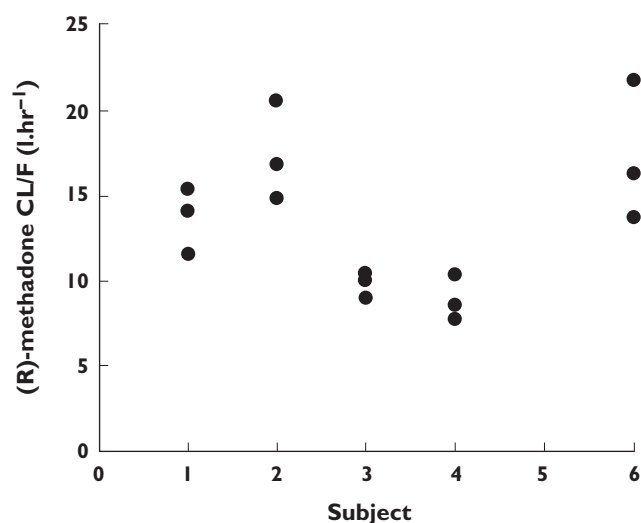


Figure 1

(R)-methadone apparent oral clearance (CL/F) in five MMT subjects on three separate occasions. The daily rac-methadone doses were 20 mg (subject 1), 65 mg (subject 2), 140 mg (subject 3), 105 mg (subject 4), and 160 mg (subject 6)

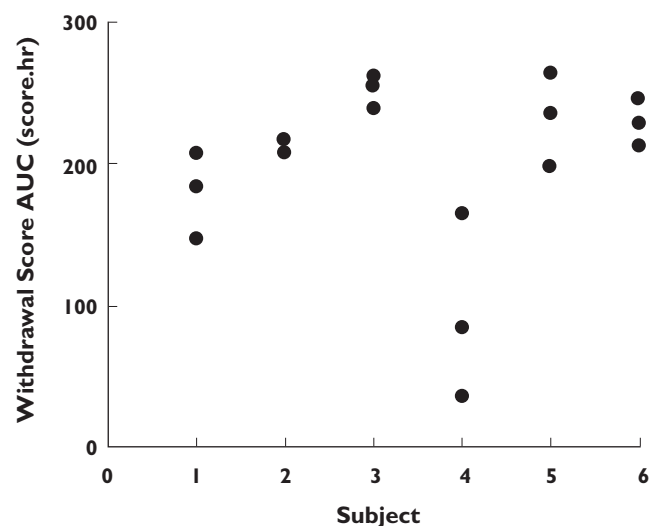


Figure 2

Area under the withdrawal score vs. time curve (AUC) over one interdosing interval in six MMT subjects on three separate occasions. The daily rac-methadone doses were 20 mg (subject 1), 65 mg (subject 2), 140 mg (subject 3), 105 mg (subject 4), 170 mg (subject 5), and 160 mg (subject 6)

mately 4-fold over the three occasions. There also appears to be little relationship between the daily racemic methadone dose, and the withdrawal score AUC. For example, patient 3, whose daily dose was 140 mg, and patient 6 whose daily dose was 160 mg, exhibited the highest withdrawal score AUC values. Furthermore, it is clear that the variation does not appear to increase as the magnitude of the score increases. This illustrates why a coefficient of variation is not an appropriate summary measure in this context, and indices of variance are much more informative. Between-subject variability was significantly ($P < 0.002$) greater (up to nearly 13-fold) than within-subject variability for all the pharmacodynamic measures (Table 1). Generally, objective pharmacodynamic effects (respiration rate, pupil size) had lower estimates of within-subject variability (<1.4-fold range of 95% CI at the mean value) compared with subjective effects, especially withdrawal (2.0-fold) and direct opioid effects (4.2-fold).

Discussion

To our knowledge, this is the first study to have investigated within-subject and between-subject variability in the pharmacokinetics and pharmacodynamics of methadone at steady state in patients on MMT. To enable a comparison of these estimates of variability, repeated measures on several occasions must be obtained. On each occasion, subject adherence (compliance) with the

dosing regimen must be complete, and drug dose and extrinsic factors governing bioavailability and clearance must remain unchanged. Our study design fulfilled these criteria. We chose nonholders because they are of particular clinical interest due to their potentially greater risk of not remaining in treatment.

The coefficient of variation is a popular summary measure of variation in experimental data, and assumes a constant proportional relationship between the standard deviation and the magnitude of the measure. Whereas this is often the case, for example in drug analysis, problems arise when there is evidence that this underlying assumption is not met, as was the case with our data. Thus, we report the between- to within-individual variance ratio, which affords a much more appropriate, reliable and informative index of variation. However, this ratio does not provide a measure of the magnitude of within-subject variability. Such information is afforded by the margin of error for the 95% confidence interval for within-subject measures. In theory, the margin of error can be applied directly to the value of any variable, as this measure is independent of the magnitude of the variable. Therefore, to provide a context for the margin of error, we calculated 95% confidence intervals using the grand mean for all variables (i.e. a 'typical value'). The degree of within-subject variability can then be assessed by inspecting the width of the 95% confidence intervals.

As postulated, within-subject variability in all end points was substantially less than between-subject variability. The latter for several pharmacokinetic parameters has been reported for patients in MMT programmes since the mid 1970s. However, most of these reports have dealt with the racemic compound and not the individual enantiomers. Values for between-subject variability at steady state in MMT patients for clearance have ranged from 1.5-fold [6] to 5-fold [39] for the racemate; 3.5-fold for the (R)-enantiomer and about 5-fold for the (S)-enantiomer [37]. Similar data have been reported in single dose studies in healthy subjects, chronic pain patients and in opioid users [18]. In a recently published population pharmacokinetic analysis in a large cohort, we reported a 7-fold range of values, with a 40% coefficient of variation for intersubject variability in (R)-methadone apparent oral clearance [5]. The range of such values reported here is consistent with previous literature, and similar variability was found for the other pharmacokinetic parameters.

There are few reports regarding within-subject variability in methadone pharmacokinetics. Eap and coworkers [40] compared the between-subject variability (about 5-fold) and within-subject variability (negligible) in the ratio of plasma (R)- to (S)-methadone concentrations in 'trough' blood samples from 20 MMT patients. Although these data provide evidence that the influence of stereoselectivity on the disposition of methadone is consistent in an individual, it does not afford any indication of the within-subject variability in methadone blood concentrations, and hence methadone clearance. In contrast, our analyses show that between-subject variability in methadone pharmacokinetic parameters is up to 8-fold greater than within subject variability. Importantly, the apparent oral clearance of the active (R)-isomer appears to be relatively consistent between occasions within an individual, as the within-subject 95% CI ranged only 1.7-fold at the mean clearance value. In contrast (S)-methadone shows greater within-subject variability (95% CI ranged 3.4-fold), which may explain the smaller between- to within-subject variance ratio estimate for (S)-methadone (2.7) compared with (R)-methadone (4.9). This may be of clinical importance as high (S)-methadone concentrations may be associated with significant adverse events [41].

Intuitively one might predict that the between-individual variability in drug disposition is greater than the within-individual variability. Examples include: alcohol [42], gabapentin [43], talinolol [44], ropivacaine [45], hydroxychloroquine [46] and etoposide [47]. It is noteworthy that there are no common features determining the disposition mechanisms for these examples.

There are other drugs, however, for which within-subject variability in drug disposition is not greatly different from between-subject variability. Examples include avitriptan [48], verapamil [49, 50], frusemide [51] and ibuprofen [52]. Our results for methadone, and those listed above, indicate that specific, well designed trials need to be conducted with each drug to determine differences in between- and within-subject variability in disposition.

In contrast to the variability reported for its pharmacokinetics, variability in the pharmacodynamics of methadone has been much less frequently studied. Boulton and coworkers [53] reported a 40% between-subject coefficient of variation in pupil response following a single dose of methadone in eight healthy subjects. In a cohort of MMT patients, Dyer and coworkers found a coefficient of variation of 75% for between-subject variability in the EC_{50} for pupil diameter [13]; a 25% value for total mood disturbance [14]; a 48% value for direct opioid effects [13]; and a 97% value for withdrawal score [13]. Inturrisi and coworkers reported a 10-fold [29] to 30-fold [30] between-subject variability in the EC_{50} for pain relief in patients with cancer being treated with a variety of opioids. One subject was studied twice over an interval of 2.5 months [29]; and the EC_{50} values on the two occasions were almost identical. Our results demonstrate that objective measures of opioid effect appear to be consistent within an individual, relative to a much greater between-subject variability. In contrast, subjective opioid effects appear to be more variable between occasions, which may explain the smaller between- to within-variance ratio estimates for subjective measures.

Despite the small numbers in this study, we have been able to show highly significant differences in the between- to within-subject variability for all of the pharmacokinetic and pharmacodynamic end points measured. Importantly, this includes both the pharmacokinetic parameters, and both objective (such as pupil diameter) and subjective (such as withdrawal) pharmacodynamic measures. There is a reasonable understanding of factors that might influence variability in methadone disposition. These include hepatic CYP3A4 activity and the presence of interacting comedications. In contrast, factors influencing variability in pharmacodynamics are poorly defined. However, it is likely that pharmacogenomics play a central role, as methadone is a p-glycoprotein substrate, genetic variations of which will affect the brain distribution kinetics of methadone, and variations in the genes for the μ opioid receptor, its G-proteins and transduction elements contribute to variability in the

response to opioid drugs [54]. Despite this, we have shown a striking difference in the between- to within-subject variability in both subjective measures, such as withdrawal, positive opioid effects and pain sensation, and more objective measures such as pupil diameter and respiration rate. This suggests that, in this population, these end-points are likely to provide a clinically useful guide to treatment.

Four subjects were receiving daily methadone doses in excess of 100 mg. This observation is consistent with our previously reported findings that many non-holders are taking relatively high doses of methadone [12, 13]. As a result, our data suggest that a nonholder taking a relatively high dose on one occasion is more likely than not to behave as a nonholder on subsequent occasions. We predict that the same would apply to a holder, although this would need to be confirmed.

Our findings have several clinical implications. If therapeutic drug monitoring is employed and blood is collected just before the next dose (C_{\min}), the clinician can be reassured that the result will be reasonably consistent from occasion to occasion over a 1–2 month period, as long as the dose and adherence remain unchanged. Our data suggest that an individual experiences fairly consistent pharmacodynamic effects from occasion to occasion on a constant treatment regimen. This is especially the case for objective effects, and the subjective effects, which provide an index of mood disturbance, pain tolerance and, most importantly, withdrawal severity. Therefore, identification of holder/nonholder status early in the course of MMT might influence the choice of the most appropriate substitution drug. In this case, an alternative to methadone, such as buprenorphine [34], or slow release oral morphine [15] might be more appropriate. This may translate into improved retention in maintenance programs and a greater likelihood of a better clinical outcome.

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